trabeculae. To assess cTnC-cTnI interactions that may underlie this functional effect, we have used both solution and modeling approaches. In solution, cTnC affinity for cTnI was assessed by spectrofluorimetry via labeling cTnC (C35S) with IANBD at Cys84. In both the absence and presence of Ca2+, C35S, L48Q cTnC had increased affinity for cTnI while C35S, I61Q cTnC (and other variants) had reduced affinity. To examine this in more detail we studied the molecular interactions between the (+/-Ca2+)-cTnC1-89-cTnI147-163 complex using molecular dynamics (MD) simulations. Multiple MD simulations (~ 100ns) of wild-type (WT) and the cTnC variants were performed to predict specific structural effects of each residue substitution. Results showed the 48 position of WT and the variant positions of cTnC had the most intermolecular contact pairs with cTnI(147-163). L48Q greatly increased time of contact with cTnI hydrophobic residues Ile148, Met153 and Leu157 compared with WT complex. At ~45ns simulation the B-helix of L48Q cTnC1-89 ''lifted", suggesting a move favorable ''opening up'' of the N-lobe hydrophobic patch compared with WT cTnC1-89. This could lead to stronger binding with the regulatory region of cTnI. Thus, our computational results provide novel details about specific structural alterations throughout L48Q cTnC and other cTnC variants. NIH-HL65497 (MR), AHA-09PRE2090056 (DW)

773-Pos

Structure of the Regulatory Domain of Human Cardiac Troponin C in Complex with the Switch Region of Cardiac Troponin I and the Drug W7: The Basis of W7 as an Inhibitor of Cardiac Muscle Contraction

Marta Oleszczuk, Ian M. Robertson, Monica X. Li, Brian D. Sykes.
University of Alberta, Edmonton, AB, Canada.

The solution structure of Ca^{2+}-bound regulatory domain of cardiac troponin C (cNTnC) in complex with the switch region of troponin I ($cTnI_{147-163}$) and the calmodulin antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfinamide (W7), has been determined by NMR spectroscopy. The structure reveals that the W7 chloronaphthalene ring interacts with the terminal methyl groups of M47, M60, and M81 as well as aliphatic and aromatic side-chains of several other residues in the hydrophobic pocket of cNTnC, while the N-(6-aminohexyl) tail interacts with the C- and D-helices of cNTnC and with $cTnI_{147-163}$. Compared to the structure of the cNTnC•Ca^{2+}•W7 complex (Hoffman, R. M. B. and Sykes, B. D. (2009) *Biochemistry* 48, 5541-5552), the tail of W7 moves toward the surface of cNTnC, in close proximity to the N-terminus of $cTnI_{147-163}$. As a result, the N-terminus of the peptide clashes with the positively charged amino group of the W7 molecule and this repulsive interaction diminishes the helical content of $cTnI_{147-163}$ when compared to the structure of cNTnC•Ca^{2+}•$cTnI_{147-163}$ (Li, M. X., Spyracopoulos, L., and Sykes B. D. (1999) *Biochemistry* 38, 8289-8298). Thus the ternary structure cNTnC•Ca^{2+}•W7•$cTnI_{147-163}$ reported in this study provides a structural basis for the inhibitory effect of W7 in cardiac muscle contraction. The structure also offers an explanation for the ~10-fold affinity reduction of $cTnI_{147-163}$ for cNTnC•Ca^{2+} in the presence of W7. This result generates insights into the features that are useful for the design of cTnC-specific Ca^{2+}-desensitizing drugs.

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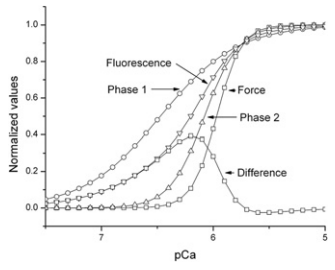
A Second Look at the Two Phases of Ca^{2+} Binding to Fast Skinned Fibers

Philip W. Brandt[1], **Corrado Poggesi**[2].
[1]Columbia University, New York, NY, USA, [2]Università di Firenze, Firenze, Italy.

Experiments that replace native TnC with TnC_{danz} in skinned rabbit psoas muscle fibers generate pCa/Ca^{2+} binding curves that are left of and roughly parallel to the pCa/force curve (Guth & Potter, 1987). The fluorescence curve best fits two binding phases, #1 with a slope of about one and #2 with a slope of about 3 or more, a cooperative binding that is associated with major force development (Allen et al, 1992, Huang et al, 2001). Phase 1 is also accompanied with subtle increments in force. The force, fluorescence, phase 1 & 2 curves synthesized from mean fitted parameters for 22 experiments are shown. To demonstrate the difference between force and binding, we subtract the force from the fluorescence. The difference curve indicates that phase 1 Ca^{2+} binding dominates at high pCa then decreases as cooperative binding increases. We argue that the regulatory sites are shifting from normal to cooperative binding and this is why the phase 2 parameters can only approximate force. Because phase 2 binding begins on top of phase 1 the fluorescence curve is shifted left away from force.



775-Pos

Effect of D145E Mutation on Calcium Binding and Exchange with the C-Domain of Troponin C

Svetlana Tikunova, Nicholas G. Swindle.
University of Houston, Houston, TX, USA.

Recent discoveries of a number of hypertrophic cardiomyopathy related mutations in the C-terminal domain of cardiac troponin C suggest that sites III and IV might play a more important role than just anchoring troponin C into the troponin complex. We investigated the effects of hypertrophic cardiomyopathy related mutation D145E in human cardiac troponin C on calcium binding and exchange with the C-terminal domain sites III and IV. The calcium titration data indicated that the D145E substitution in the +z position of the calcium binding site IV dramatically decreased calcium binding affinity of that site (~1, 856-fold), and virtually eliminated magnesium binding to that site. Furthermore, the D145E substitution significantly decreased the calcium affinity of site III (~1.4-fold), correlating with ~1.6-fold faster rate of calcium dissociation from site III. Stopped-flow studies utilizing fluorescent calcium chelator Quin-2 demonstrated that the D145E mutation reduced the stoichiometry of moles of calcium per mole of the C-terminal domain by ~2-fold, both in the absence and presence of cardiac troponin I peptide (residues 34-71). Thus, binding of troponin I peptide to the C-terminal domain of D145E troponin C was not able to restore normal calcium binding to site IV. These results indicate the conservative D145E substitution has detrimental effects on calcium and magnesium binding to site IV of troponin C.

776-Pos

Effect Of Down-Regulation of a Stretch-Activated TnC Isoform on Flight of Drosophila

Anja Katzemich, Friederika Thiele, **Belinda Bullard**.
University of York, York, United Kingdom.

Both *Drosophila* and *Lethocerus* have indirect flight muscle (IFM) that is activated by sinusoidal length changes at constant [Ca^{2+}]. IFM has two TnC isoforms. F1 binds a single Ca^{2+} in the C-lobe and is needed for the periodic stretch-activation of fibres to produce oscillatory work. F2 binds Ca^{2+} in both N- and C-lobes and is needed for producing Ca^{2+}-dependent isometric tension. We have obtained flies (from VDRC, Vienna) in which F1 is down-regulated by RNAi. Male flies of the F1 RNAi line were crossed with virgin female flies having the Dmef2 driver, which is expressed in all muscles, or a UH3 driver, which is expressed only in IFM. Crosses were maintained at 25°C and 29°C to get different levels of RNAi expression. The proportion of flies unable to fly was: wt 0%; Dmef2 87% at 25°C, 90% at 29°C; UH3 70% at 25°C, 100% at 29°C. There was no difference in time of development or viability of the different lines. Confocal microscopy of Dmef2 and UH3 flies showed myofibrils of both lines were narrower than wt; sarcomere length was normal, but Z-disc and M-line were not straight. Electron microscopy showed that sarcomere structure was disrupted more than expected. Troponin was regularly spaced at 38 nm along thin filaments, but thick and thin filaments were misaligned and Z-and M-lines shifted. Blots of IFM with anti-F1 and F2 showed F1was absent in Dmef2 flies, and greatly reduced in UH3 flies; F2 content of IFM was the same as wt. Therefore, F1 is essential for maintaining normal sarcomere structure of IFM, as well as for stretch-activation. Evidence for cross-linking between troponin components and thick filaments of *Lethocerus* IFM will be presented. Lack of F1 may affect these links.

777-Pos

The Effect of Glutathione on Skeletal Muscle Calcium Sensitivity and Myofilament Sulfhydryl Groups

Sean Gross, Steven Lehman.
UC Berkeley, Berkeley, CA, USA.

Glutathione, a critical reducing agent present in relatively high levels(~5mM) in skeletal muscle, can also attach to protein thiols via a disulfide bond. This process is referred to as glutathionylation, and is thought to be a protective mechanism to prevent irreversible protein oxidation. Prior studies have shown that when skinned fibers are exposed to reduced glutathione there is an increase in calcium sensitivity with no significant change in maximal force. These calcium sensitivity changes were largely reversible by the reducing agent DTT, indicating modification of protein thiols. We measured the force-pCa relationship of permeabilized rabbit psoas fibers treated with DTDP a thiol-specific oxidizing agent and glutathione (5mM). 2D gel electrophoresis using either IEF 4-6.5 or NEPHGE 3-10 in the first dimension was used to identify myofilament proteins whose sulfhydryl groups were modified with the oxidant-glutathione treatment. Additionally, phosphorylation of the regulatory myosin light chain was analyzed using 2d gels (IEF 4-6.5) and the phosphorylation specific stain Diamond Pro-Q. The pCa 50 of skinned psoas fibers was decreased upon exposure to DTT. Following DTT, the addition of DTDP and GSH sequentially,